Vol. 3. No. 2. June, 1946.

THE QUEENSLAND JOURNAL OF AGRICULTURAL SCIENCE

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PUBLISHED QUARTERLY

By the Queensland Department of Agriculture and Stock, Brisbane

Issued by Direction of Hon. H. H. COLLINS,
Secretary for Agriculture and Stock.

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THE QUEENSLAND JOURNAL OF AGRICULTURAL SCIENCE

VOL. 3. NO. 2.

JUNE, 1946

EXPERIMENTS IN THE CONTROL OF BACTERIAL WILT OF TOMATOES IN SOUTH-EASTERN QUEENSLAND.

By J. E. C. ABERDEEN, B.Sc.Agr., Pathologist, Science Branch, Division of Plant Industry.

SUMMARY.

1. Under the conditions of the experiment, treatment of the soil with sulphur to produce a pH value of 4, followed by the addition of lime to restore the pH value to 5, failed to give efficient control of bacterial wilt in tomatoes.

2. Varietal trials on infested soil showed that Break o' Day, which is widely grown locally, was very susceptible to the disease. Strains of another Australian variety, Sensation, and several varieties introduced from the United States of America, showed promise of possessing some degree of resistance.

INTRODUCTION.

Bacterial wilt of tomatoes, caused by *Xanthomonas solanacearum* (E.F.Sm.) Dowson, is most apparent in south-eastern Queensland during February and March, i.e., in the latter half of summer. Growers wishing to obtain an early autumn crop, which commands good prices on the local market, commence planting in January, and it is this crop which is often seriously affected by the disease.

The problem of control was approached from two angles, viz., soil treatment to eliminate the causal bacteria, and the selection of varieties or strains resistant to the disease. The work was interrupted owing to war service, but it is considered that the results obtained, and particularly the data on the degree of resistance of the different varieties, may be of use to other workers on this problem.

SOIL TREATMENT.

The soil treatments given followed the principle of the experiments of A. H. Eddins in Florida (Eddins, 1936, 1938, 1939). This worker applied sulphur to the soil at a rate estimated to be sufficient to reduce the final pH value to 4 or slightly less; the soil was then allowed to stand for a period of time,

after which it was limed to restore the pH value to approximately 5. In the successful Florida experiments, the sulphur was applied about midsummer and the ground lime approximately five months later. No mention was made in Eddin's papers of the time at which the field was planted, but probably it would be in the following spring, several months after liming. The period of high acidity during the five months which elapsed between the application of sulphur and liming was apparently sufficient to destroy, or to render non-virulent, the causal organism, as Eddins had considerable success in the control of bacterial wilt in potatoes, egg plant and tomatoes.

To follow Eddins' seasonal procedure in south-eastern Queensland would mean applying the sulphur in January and the lime in June. However, because of the local cropping programme it was considered that it would be more convenient if the sulphur was applied in autumn to early winter and the lime in midsummer, and the plants put out into the field about a month after liming.

Two areas known to be infected with *X. solanacearum* were treated in the above fashion for two years in succession. For convenience of discussion, the areas will be designated A and B. The soil in area A was a clay loam of a dark grey-brown to dark-brown colour, while that in area B was a sandy loam to loam of a light red-brown colour. The initial pH values for the two areas varied from 5·9 to 6·1 and 5·2 to 5·4 respectively, and sulphur at the rate of 2,850 lb. and 1,520 lb. per acre was required in the first season to reduce the pH value to approximately 4. To raise the pH value to 5, hydrated lime was later applied at the rate of 2,620 lb. and 1,750 lb. per acre respectively to A and B. The same plots were again sulphured and limed in the following season; the pH values were 5·1–5·5 and 4·7–4·8 for areas A and B respectively prior to sulphuring, and consequently less sulphur was required than in the first season. On area A the experiment was laid out as six randomized blocks and on area B as four randomized blocks. In the 1938-39 trials, Break o' Day variety only was used, but in the 1939-40 trials a number of varieties was planted in order to obtain some lead on varietal resistance to bacterial wilt.

There was a period of $3\frac{1}{2}$ months between sulphuring and liming in the 1938-39 season and of 7 months in 1939-40. However, in both seasons and on both areas the actual period for which the pH values were approximately 4 was only a month, as a very small drop in pH values took place during the winter months.

The only result obtained from the soil treatments was a delay in the rate of incidence of the disease on the sulphured and limed plots. There was a final mortality of practically 100 per cent. in all plots of Break o' Day before even the earliest maturing fruit could be harvested. This is in accord with the results reported by Eddins (1938) for sulphur applications made in the autumn or winter. A trial providing for summer application of sulphur was commenced but was abandoned because of war conditions.

VARIETAL RESISTANCE.

The first indication in Queensland that a variety might be relatively resistant to the strains of *X. solanacearum* present here was afforded by the growing of several rows of the variety Sensation adjacent to a badly infected crop of Break o' Day on one of the experiment plots in the summer of 1938-39. In order to pursue this aspect of the investigation further, the 1939-40 soil treatment experiments were planted with a number of varieties. From this preliminary experiment, it was evident that Break o' Day is very susceptible to the disease. Though there was not sufficient evidence from which to draw any definite conclusions, the varieties Sensation, Australian Earliana, Marvana and Rutgers showed indications of possessing some resistance to bacterial wilt.

During the summer of 1940-41, the two blocks used previously for the investigation of soil treatments were planted to a variety trial without any further differential soil treatments. One area was planted with 10 varieties and strains, as four randomized blocks, and the other with 20 varieties as three randomized blocks. The arrangement of the blocks was such that the previous year's soil treatments did not invalidate the statistical analysis of the varietal experiment. Seedlings were planted in the field on January 28-29, 1941 and any misses were replaced on February 6 and 12. Growth was excellent over both plots until the appearance of the wilt.

Table 1.

VARIETAL RESISTANCE TO BACTERIAL WILT.
(Summary of Mean Index Values).

Variety.	Mean Index.	Significantly exceeds	
		5 per cent. level.	1 per cent. level.
1. Sensation No. 2	3·57	9–16	11–16
2. Sensation No. 1	3·13	11–16	12–16
3. Marvel	2·97	11–16	13–16
4. Sensation No. 3	2·77	11–16	13–16
5. Marvana No. 1	2·67	12–16	13–16
6. Marvana No. 2	2·57	13–16	14–16
7. Denisonia	2·40	13–16	16
8. Homer	2·37	13–16	16
9. Bowen Buckeye	2·30	13–16	16
10. Australian Earliana	2·27	13–16	16
11. Rutgers No. 1	1·50	..	..
12. Rutgers No. 2	1·40	..	..
13. Break o' Day No. 1	·93	..	..
14. Break o' Day No. 4	·83	..	..
15. Break o' Day No. 2	·80	..	..
16. Break o' Day No. 3	·60	..	..

Necessary difference for significance 5% level = 1·23 ;
1% level = 1·66.

As a rapid means of estimating wilt resistance, each plant was given a numerical value according to the following system:—

0 = a plant that is dead, or extremely stunted.

1 = a plant severely wilted, but still green.

2 = a plant with only one or two branches wilted.

3 = a plant with only one leaf or several leaflets showing wilt.

4 = a plant apparently healthy and of fair growth.

5 = a plant apparently healthy and of vigorous growth.

The results obtained from the two trials are given in Tables 1 and 2.

Table 2.

VARIETAL RESISTANCE TO BACTERIAL WILT.
(Summary of Mean Index Values).

Variety.	Mean Index.	Significantly exceeds	
		5 per cent. level.	1 per cent. level.
1. Sensation No. 1	3·87	8–20	11–20
2. Sensation No. 2	3·80	8–20	11–20
3. Sensation No. 3	3·60	11–20	13–20
4. Marvel	3·13	14–20	14–20
5. Carolina Cross No. 2	3·13	14–20	14–20
6. Louisana Pink No. 1	3·07	14–20	14–20
7. Bowen Buckeye	2·93	14–20	14–20
8. Prairiana	2·70	14–20	16–20
9. Louisana Pink No. 2	2·67	14–20	16–20
10. Marvana No. 1	2·63	14–20	17–20
11. Australian Earliana	2·27	17–20	19–20
12. Carolina Cross No. 1	2·23	17–20	20
13. Rutgers No. 1	2·13	17–20	20
14. Rutgers No. 2	1·40	..	..
15. *Lycopersicon pimpinellifolium*	1·33	..	..
16. Denisonia	1·23	..	..
17. Break o' Day No. 1	1·03	..	..
18. Break o' Day No. 4	·93	..	..
19. Break o' Day No. 2	·83	..	..
20. Break o' Day No. 3	·60	..	..

Necessary difference for significance 5% level = 1·06;
1% level = 1·41.

A progressive estimate of wilt values was made at weekly intervals and the estimates as made on April 4 and 8 were used for final comparisons and analysis. Each plant was given an individual value and from these a mean plot value was estimated.

DISCUSSION.

The extreme susceptibility of Break o' Day is apparent, while Sensation and Marvel gave quite encouraging results in both trials. Louisiana Pink No. 1 and Carolina Cross No. 2 also gave good results in the trials in which they were included.

Sensation is not, however, a good commercial variety, though it is a vigorous grower and the fruit externally has an excellent appearance. It has the disadvantages of requiring trellissing and pruning to assist development of fruit size and of possessing unsatisfactory internal fruit characteristics.

Louisiana Pink, Prairiana and the Carolina Cross were received from the North Carolina Agricultural Experiment Station, in January, 1940, and had already been selected for resistance to bacterial wilt in that State. The fruit type in these strains is a definite improvement on Sensation, but here also further improvement would have to be effected before the strains could take a place on the commercial market in Queensland.

Marvel was obtained from the United States Department of Agriculture; so far as is known it had not been selected for bacterial wilt resistance.

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DISTRIBUTION OF BEETLES OF THE GENUS *TRIBOLIUM* (COLEOPTERA: TENEBRIONIDAE) IN STORED FOODSTUFFS IN QUEENSLAND.

By N. E. H. CALDWELL, M.Sc.Agr., and A. R. BRIMBLECOMBE, M.Sc., Entomologists, Science Branch, Division of Plant Industry.

During an investigation of stored foodstuff pests in Queensland in 1942 and 1943, two species of the genus *Tribolium*—*T. castaneum* Herbst., the rust red flour beetle, and *T. confusum* J. du V., the confused flour beetle—were commonly encountered. The distribution of these two insects differed to some extent from that in the southern States of Australia, where *T. castaneum* is the more important pest of wheat grain, while *T. confusum* alone occurs in flour mill machinery (Miller, 1944) and predominates in flour (Miller, 1946; Nicholson, 1942).

DISTRIBUTION IN QUEENSLAND.

Both species were present in wheat stored at Queensland flour mills, which are all situated in the southern part of the State, but *T. castaneum* far outnumbered *T. confusum* in the grain samples examined. As would be expected, *T. castaneum* was also more prevalent in the wheat cleaning machinery at the mills. Within the actual milling machinery *T. confusum* was the dominant species. It occurred commonly in the nine mills examined, sometimes in high populations, whereas *T. castaneum* was found only twice in milling machinery and then in very small numbers. Samples of spilled flour and sweepings from the flour storage section of mill premises contained the two species in approximately equal numbers.

Observations in southern Queensland in environments other than flour mill premises, though less extensive than in the north, indicated that both species were quite common in warehouses and depots containing cereals and cereal products. In storage sheds, commercial stores, and other places in tropical Queensland, however, *T. castaneum* was overwhelmingly the more numerous in a wide range of commodities, such as whole grains (wheat, maize, rice and barley), crushed and cracked grains, milled cereal products, nuts and nut meats. Large populations of this species were particularly common in the more finely milled cereal products, such as flour, bran and pollard. Although *T. confusum* was comparatively rare, fairly large populations were occasionally found in flour; of the other products examined, wheat, cracked maize, pollard and oatmeal were sometimes infested.

Interesting differences in the distribution of the two species in white flour from different sources were noted in northern Queensland. Data from a typical series of uniform samples of Queensland-milled flour and from all the samples of New South Wales flour examined are summarized in Table 1.

Table 1.

TRIBOLIUM INFESTATION OF WHITE FLOUR EXAMINED IN NORTHERN QUEENSLAND.

Origin of Flour.	Number of Samples.	T. castaneum.		T. confusum.	
		Number of Samples Infested.	Number of Adults.*	Number of Samples Infested.	Number of Adults.*
Queensland	38	36	1,803	10	65
New South Wales	13	5	59	8	304

* In the case of 6 of the Queensland and 6 of the N.S.W. samples only a portion of the population (about 50 adults) was sorted. The totals given are therefore only approximate.

As population density may be closely related to the period of storage and as the bulks from which the samples were drawn had been stored for varying lengths of time, the figures given in Table 1 for the adult population do not necessarily reflect the degree of initial infestation.

The distribution of the two species of *Tribolium* in foodstuffs in Queensland may be summarized as follows:—

(1) As a general pest of stored food products in Queensland *T. castaneum* is more common than *T. confusum*, especially in the tropical section.

(2) In Queensland, as in the southern States, *T. castaneum* is the dominant species of *Tribolium* in wheat grain.

(3) While in the southern States *T. confusum* is the more important species in both flour milling machinery and flour, in Queensland it is dominant only in the machinery, *T. castaneum* being by far the more prevalent pest of flour milled within the State.

Since *T. castaneum* is so seldom met with in flour mill machinery in Queensland it must be inferred that either the species will not thrive in such an environment or the grain stream is not an important source of the *Tribolium* populations in milling machinery.

The relative scarcity of *T. confusum* in flour stored in the north of the State indicates that contaminated mill machinery is not an important source of *Tribolium* infestation in bagged flour. Direct evidence on this point was provided by examination of 230 samples taken from the flour packers in a mill known to be heavily infested with *T. confusum*. These samples were examined when collected and again after incubation in the laboratory for a period long enough to allow immature stages to develop; not a single specimen of *T. confusum* was recovered.

That climatic factors do not prevent the establishment of the insect in flour and other cereal products in northern Queensland is shown by the thriving nature of the *T. confusum* infestations which do occur and the fact that rapidly-multiplying laboratory colonies have been maintained for many months in that area.

Since the predominating species of *Tribolium* in wheat is identical with that occurring in Queensland-gristed flour stored in the north, it appeared probable that in some way the infestation in the grain is responsible for that in the flour. It is considered that this takes place through the medium of the containers, namely, jute sacks. In Queensland new sacks are used for transporting wheat from farm to mill. Later, after having been passed through mechanical cleaners, they are filled with flour. An examination of recently-cleaned sacks at a Queensland mill showed that all insects, particularly those in the seams, were not removed by the process. These survivors may later infest the flour.

Evidence in support of this view was supplied by examination of flour samples taken from consignments immediately the sacks were filled at the mill and again on arrival at Townsville after a rail journey of several days duration. Results of this examination are given in Table 2.

Table 2.

Tribolium Infestation of White Flour before and after Transport in Cleaned Wheat Sacks.

—	Number of Consignments Sampled.	Total Number of Samples.*	T. castaneum.		T. confusum.	
			Number of Samples Infested.	Number of All Stages.	Number of Samples Infested.	Number of All Stages.
Brisbane	6	230	1	1	0	0
Townsville	4	126	20	130	3	6

* Size of samples—8 oz. from first consignment, 4 oz. from succeeding consignments.

After an initial examination the samples were incubated for a period sufficiently long for eggs and very small larvae to reach maturity before a second examination. Risk of contamination before or after the journey was minimized by despatching the flour by rail from Brisbane as soon as practicable after packing and by sampling before unloading at Townsville. It may be assumed that infestation would not be initiated in transit, as examination of numerous railway waggons over a lengthy period revealed no specimens of either species of *Tribolium*.

The presence of *T. confusum* in Queensland-milled flour at odd times may be due to an occasional carry-over of the pest in the sacks or to the accidental contamination of the bagged product from the population in the mill machinery.

The comparative abundance of *T. confusum* in New South Wales flour suggests differences in mill practice which increase the importance of this species.

It is concluded that the occurrence of *T. confusum* and *T. castaneum* in flour in Queensland is determined by the source of initial contamination rather than by the nature of the foodstuff or by climatic or other conditions.

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INVESTIGATIONS IN THE CONTROL OF THE TOBACCO LEAF-MINER, *GNORIMOSCHEMA OPERCULELLA* ZELL. (LEPIDOPTERA: GELECHIIDAE), WITH D.D.T. AND "GAMMEXANE."

By R. C. CANNON, B.Sc.Agr., Assistant Entomologist, and N. E. H. CALDWELL, M.Sc.Agr., Entomologist, Science Branch, Division of Plant Industry.

SUMMARY.

1. Experiments in the control of the tobacco leaf-miner, Gnorimoschema operculella Zell., with D.D.T. and "Gammexane" were conducted at Mareeba in northern Queensland.

2. Effective control was obtained with 0·1 and 0·2 per cent. D.D.T. sprays and 1·0 and 2·0 per cent. D.D.T. dusts applied immediately after transplanting, followed by regular fortnightly treatments up to a maximum of five applications in all. A total of three applications made at fortnightly intervals also afforded a high degree of protection to the crop.

3. "Gammexane" at the high concentration was inferior to D.D.T. even at the low concentration of the latter; in addition, the sprays and dusts containing it had such a pronounced phytocidal effect on the tobacco plants that their use had to be discontinued before the termination of the experiment.

INTRODUCTION.

The tobacco leaf-miner, *Gnorimoschema operculella* Zell., was early recognized as a major pest of tobacco in northern Queensland. Atherton (1936) obtained only partial control with frequent applications of a 50 per cent. lead arsenate dust, while other insecticides tested proved of little value. The fact that no external feeding takes place and the larvae are thus virtually inaccessible during the period of feeding may in a large measure account for the inadequacy of these insecticides. The recent advent of D.D.T. and the promising results obtained from its use against this species in other crops prompted the investigation reported here.

SCOPE OF THE INVESTIGATION.

It had already been demonstrated in a preliminary tobacco seed-bed experiment (Caldwell, 1945) that the phytocidal effect of solvent naphtha-D.D.T.-"Wetsit"* sprays, when used at concentrations for the present adopted as standards in experimental work, was limited to a negligible degree of stunting, from which the plants rapidly recovered once applications had ceased. In the

* Sodium alkyl naphthalene sulphonate plus pine oil.

meantime, insecticides containing "Gammexane"† had become available and they were included for trial, though there had been no opportunity to test their phytocidal effect beforehand. The materials used were impregnated dusts containing D.D.T. or "Gammexane" in a pyrophyllite diluent, mayonnaise emulsions of both insecticides, and a solvent naphtha—"Wetsit" emulsion of D.D.T.

All insecticides were tested at two levels, the concentrations referred to hereunder being given in terms of the para para isomer of dichloro-diphenyl-trichloroethane and the gamma isomer of benzene hexachloride, respectively. Low levels of sprays were 0·1 per cent. for D.D.T. and 0·026 per cent.‡ for "Gammexane," and of dusts 1·0 per cent. and 0·26 per cent.‡ respectively. The high levels were in each case twice the above concentrations. Provision was made for a comparison between a total of three and a total of five regular applications at fortnightly intervals, commencing immediately after the seedlings were transplanted into the field.

EXPERIMENTAL DETAILS.

Crop Conditions and Insect Incidence.

The experiment was conducted at Paddy's Green, Mareeba, where conditions are typical of the dry-farmed areas of the district. The technique of planting was such that four days were occupied in planting out the experimental crop of approximately two acres. The operation was restricted to the afternoons and extended from November 26 to November 29, 1945. The seedlings were of the variety Virginia Bright Leaf and were healthy and fairly uniform in size and vigour. Weather conditions subsequent to planting were favourable, resulting in a good stand, and the crop made satisfactory progress throughout, with harvesting commencing in the middle of February.

At the time of planting, the seedlings were virtually free of leaf-miner but infestation developed later to a point where, a little over one month after planting, 80 per cent. of the plants in untreated plots were infested. In the middle of January moths were observed to be again active in the crop and fresh mining was in evidence at the third sampling which commenced on February 2.

Design and Layout.

The experimental design involved a split-plot arrangement with three absolute replications. The main-plots were set out as randomized blocks and the four main-plot treatments comprised the two insecticide levels and the two

† Supplied by Imperial Chemical Industries of Australia and New Zealand Ltd. as a mayonnaise emulsion (E.F. 488M) containing 1·95 per cent. of the gamma isomer of benzene hexachloride together with 7·9 per cent. of the less active isomers—alpha, beta and delta—in an aromatic solvent, and as an impregnated dust with pyrophyllite as the diluent.

‡ The original concentrations were intended as 0·02 per cent. for sprays and 0·2 per cent. for dusts, based on a gamma benzene hexachloride content of 10 per cent. for the crude product (D 919) used in the preparation of sprays and dusts. It has since been established that the gamma isomer content of the crude product was 13 per cent. and adjustments have been made accordingly.

durations of treatment factorially arranged. The main-plots were subdivided into six sub-plots each, including the five insecticidal treatments and the control, these being randomized within the main-plots. Sub-plots were 12 feet (3 rows) wide and 90 feet long, with plants spaced 2 feet apart in the rows. Six sub-plots end to end, and separated by buffer strips 12 feet wide, constituted a main-plot, while four adjacent main-plots formed a complete block, of which there were three in this experiment.

Insecticide Applications and Treatment Modifications.

Details of insecticide applications are given below:—

Application.	Date of Application.	Number of Days since Previous Application.
First	Nov. 26–29	..
Second	Dec. 10	11–14
Third	Dec. 21	11
Fourth	Jan. 5–6	15–16
Fifth	Jan. 19	13–14

The initial applications were made each day immediately after planting and were thus spread over the four consecutive planting days. The third application was made under rather bad weather conditions owing to several showers of rain falling during the day. The fourth and fifth applications were limited to the long-term schedule plots as provided in the original design of the experiment.

Observations prior to the fourth application revealed pronounced phytocidal effects of the "Gammexane" treatments. Consequently, all but the low level of "Gammexane" dust were discontinued and a low level "Gammexane" dust substituted for the high level "Gammexane" spray and dust, and a low level D.D.T. dust for the low level "Gammexane" spray. Prior to the fifth application it was noted that all "Gammexane"-treated plots were showing severe damage by leaf-miner and leaf-eating species, such as *Heliothis armigera* Hbn., *Plusia chalcites* Esp., *Prodenia litura F.*, and Tettigoniidae, and it was decided to replace all of the original "Gammexane" treatments with corresponding levels and forms of D.D.T. so as to avoid undue crop losses.

The first treatments were applied with small hand atomizers and dusters; for subsequent applications knapsack sprayers and bellows type dusters were used. Throughout the experiment separate machines were reserved for use with D.D.T. and "Gammexane." Rates of application ranged from 24 to 36 gallons per acre for sprays and 12 to 48 pounds per acre for dusts, depending mainly on the size of the plants.

PHYTOCIDAL EFFECTS.

Reference has already been made to earlier phytocidal tests with D.D.T. wherein it was found that concentrations within the limits of those employed in this investigation were for all practical purposes innocuous. In this trial there was no evidence of any phytocidal effect with D.D.T. treatments.

The phytocidal effect of "Gammexane" sprays and dusts was most pronounced and was accentuated at the higher concentrations. There was a marked stunting of the plants with inhibition of terminal growth resulting in prolific sucker development, sometimes giving a "rosetted" appearance. Leaves were malformed and reduced in size, the development of one half of the lamina frequently being retarded or even inhibited. Accompanying these symptoms there was distinct coarsening of the leaf and roughening of the surface, accompanied by mottling. The general appearance of affected plants was reminiscent of extreme "frenching."

SAMPLING TECHNIQUE AND ASSESSMENT OF RESULTS.

Assessment of leaf-miner infestation for the purposes of the experiment was based on samples of 30 plants per sub-plot, the data being reduced to a "per plant" basis. To reduce errors due to wind drift of insecticides the two outer rows of each plot were regarded as guard rows and the sampling was taken from the inner row only. The first sampling was carried out over the period January 4-6; that is, at the time of the fourth insecticide application and thus about six weeks after the initial treatment. Two further samplings were made at approximately fortnightly intervals, on January 18-20 and on February 2-3. The same method of progression through the plots was followed at each sampling so that, except where occasional plant losses had occurred between samplings, the same plants were examined on each occasion.

Two methods of assessment were followed, namely, (*a*) a count of leaves mined and (*b*) a count, or more correctly an estimate, of the number of mines present. The former constitutes a rather severe criterion in that it takes no account of the degree of infestation in each leaf. The latter is considered to give a better estimate of larval population and severity of leaf damage but is subject to some limitations. In heavy infestations it may happen that a large mine would contain several larvae. On the other hand, a single larva may occasionally produce more than one mine when forced to vacate the original mine through accidental circumstances, such as death of the leaf in which it is feeding.

DISCUSSION OF RESULTS.

Table 1 sets out in a summarized form the data relevant to the main effects of the treatments. In view of the modifications introduced into the "Gammexane" schedule the data from these plots were omitted from the analyses except in the case of insecticide comparisons at the first sampling. For completeness and as indicative of trends the data from the first sampling are retained to show the relative merits of D.D.T. and "Gammexane" as insecticides for tobacco leaf-miner control.

Table 1.

LEAF-MINER INFESTATION (EXPRESSED AS MEANS PER PLANT).

Treatment.	Leaves Mined.			Number of Mines.		
	1st Sampling Approx. 6 Weeks after Planting.	2nd Sampling Approx. 8 Weeks after Planting.	3rd Sampling Approx. 10 Weeks after Planting.	1st Sampling Approx. 6 Weeks after Planting.	2nd Sampling Approx. 8 Weeks after Planting.	3rd Sampling Approx. 10 Weeks after Planting.
Control	1·867	2·078	3·044	3·162	4·976	7·106
3 Applications	..	0·029	0·921	..	0·038	1·389
5 Applications	..	0·007	0·134	..	0·007	0·178
Low Level	0·015	0·027	0·622	0·021	0·032	0·960
High Level	0·000	0·009	0·434	0·000	0·013	0·607
Differences for Significance 5% ..	0·063	0·031	0·363	0·068	0·043	0·648
Differences for Significance 1% ..	0·091	0·042	0·550	0·091	0·059	0·982
D.D.T. S/N* Spray ..	0·000	0·005	0·543	0·000	0·005	0·803
D.D.T. M/E.† Spray ..	0·000	0·002	0·242	0·000	0·002	0·334
D.D.T. Dust	0·022	0·046	0·798	0·032	0·060	1·213
Gammexane M/E.†Spray	0·188	..	..	0·257	..	..
Gammexane Dust ..	0·429	..	..	0·588	..	..
Differences for Significance 5% ..	0·076	0·038	0·149	0·108	0·053	0·290
Differences for Significance 1% ..	0·102	0·051	0·206	0·144	0·072	0·406

* S/N = solvent naphtha—" Wetsit " emulsion.

† M/E. = mayonnaise emulsion.

The effect of duration of treatment can be seen by reference to the data from the second and third samplings. It is evident that three applications of D.D.T. sprays and dusts at approximately fortnightly intervals from transplanting did not afford adequate protection during the whole of the post-treatment period. The absence of differences of any magnitude at the second sampling, made four weeks after the third application, indicates that the residual effect of these insecticides persists for approximately this period, but the fact that there was a slight increase in infestation at this time points to one month as the upper limit of persistency. From this evidence it may be inferred that the fifth application, in view of the approach of harvesting, was probably superfluous.

On the above grounds it would appear that fortnightly intervals between treatments, as adopted in this experiment, were unnecessarily short. There is, however, the question of coverage of new growth to be considered. This assumes particular importance with a crop such as tobacco, in which the surface area of the foliage increases rapidly during the earlier stages of growth. For this reason monthly applications might in practice prove too infrequent to ensure adequate protection under all conditions.

When the data for "Gammexane" treatments at the first sampling were included in the estimates of main-plot effects, a significant difference between the two levels occurred, due to the marked significant difference between the higher and lower concentrations of "Gammexane," as shown in Table 2.

Table 2.

LEAF-MINER INFESTATION AT FIRST SAMPLING EXPRESSED AS MEANS PER PLANT.

Treatment.	Leaves Mined.		Number of Mines.	
	Low Level.	High Level.	Low Level.	High Level.
D.D.T. S/N Spray	0·000	0·000	0·000	0·000
D.D.T. M/E Spray	0·000	0·000	0·000	0·000
D.D.T. Dust	0·045	0·000	0·063	0·000
Gammexane M/E Spray	0·243	0·133	0·357	0·157
Gammexane Dust	0·688	0·190	0·943	0·233
Mean	0·191	0·065	0·273	0·078

The first sampling showed that "Gammexane" exercised a considerable degree of control over the leaf-miner but that it was obviously inferior to D.D.T. at the concentrations used. Thereafter any strict comparison between the two insecticides was rendered impossible by virtue of the treatment modifications which were introduced. In any case the phytocidal effect of the "Gammexane" sprays and dusts would preclude their use on tobacco, at least in the forms used in this experiment.

D.D.T. was outstandingly superior to the controls. The data on types and forms of insecticides set out in Table 1 are means of long and short term treatments. Table 3 sets out the data relevant to D.D.T. in the series of long duration only, from which it is possible to compare sprays and dusts without any masking effect from the less adequate short term treatments.

Table 3.

LEAF-MINER INFESTATION WITH 5 D.D.T. APPLICATIONS EXPRESSED AS MEANS PER PLANT.

Form in which D.D.T. Applied.	Leaves Mined.			Number of Mines.		
	1st Sampling.	2nd Sampling.	3rd Sampling.	1st Sampling.	2nd Sampling.	3rd Sampling.
S/N Spray	0·000	0·000	0·020	0·000	0·000	0·020
M/E Spray	0·000	0·000	0·017	0·000	0·000	0·017
Dust	0·022	0·020	0·367	0·032	0·020	0·498
Differences for Significance 5%	0·107	0·054	0·211	0·153	0·075	0·410
Differences for Significance 1%	0·144	0·072	0·291	0·204	0·102	0·566

From Table 3 it is clear that there is no significant difference between the two types of spray, but there is a consistent difference in favour of sprays as against the dust, this difference being significant at the third sampling. In comparing the efficacy of sprays and dusts one must not lose sight of the fact that they are applied by different types of machine, variations in the efficiency of which might well lead to slightly different results. Another point is that by the time of the fourth and fifth applications the crop had made considerable growth and treatments were for several reasons concentrated on the upper portions of the plants. It is practically certain that leaf-miner larvae in the lower leaves would not have come within the sphere of influence of the fifth insecticidal application; thus the difference in favour of the sprays may be related to a more pronounced residual effect of the latter as compared with the dust.

CONCLUSIONS.

The results of this investigation make it clear that a high degree of control of the leaf-miner in tobacco may be obtained by the routine application of D.D.T. sprays or dusts in the field. The evidence points to concentrations of 0·1 per cent. of the para para isomer of dichloro-diphenyl-trichloroethane for sprays and 1·0 per cent. for dusts as being adequate for this purpose. Protection should be afforded by an application of D.D.T. either immediately before or immediately after the seedlings are transplanted, to be followed by two or three further treatments at intervals of two weeks or a little more. The initial application could be most cheaply and effectively applied to the seed-beds prior to planting out. Treatment along these lines can be expected to control the pest when relatively high populations occur in crops subject to reasonably favourable growing conditions.

In this experiment, the seedlings were virtually free from leaf-miner when transplanted. The pest can, however, be a source of considerable injury in the seed-beds and it may be inferred from the investigation outlined above that applications of a D.D.T. spray or dust if and when necessary will control the pest in the seed-bed also.

ACKNOWLEDGEMENTS.

Statistical analyses of the data have been carried out by Mr. P. B. McGovern (Biometrician). It is desired to acknowledge the assistance of Mr. E. W. Baird, Adviser in Agriculture at Mareeba, in the field work associated with the experiment. Thanks are also due to Messrs. Maingay and Langley, on whose farm the work was carried out.

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A METHOD FOR DETECTING SOURCES OF MILK CONTAMINATION ON FARMS.

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SUMMARY.

A method has been devised for locating readily the sources of milk contamination on dairy farms. The method embodies rinsing with aseptically drawn milk all surfaces likely to provide contamination and performing bacteriological tests on the rinsings. It has been found practicable under Queensland field conditions, and, furthermore, lends itself to considerable modification to suit various conditions.

Results of methylene blue reduction tests and plate counts for several farms are tabulated.

INTRODUCTION.

Experience gained in the control of the bacteriological quality of raw milk supplies has shown that the mere notification of low quality to farmers often does not lead to any real improvement in milk quality. Greater benefit results from instructional visits by dairy advisory officers to farms supplying low quality milk, which are usually successful in effecting improvement in quality. Unfortunately, however, there are many instances where well equipped and apparently well managed dairies produce milk with a low methylene blue reduction time and which shows evidence on microscopical examination of considerable bacterial contamination. In such cases the equipment appears to be superficially clean, and often no fault can be found with the methods of milk production. A method for accurately locating foci of contamination on the farm has been devised which is particularly useful in instances where the cause of low reduction times is not apparent on visual examination.

The method was designed primarily for use in connexion with experimental work concerned with the farm contamination of raw milk supplies, but it is considered that it can be applied by advisory officers as a normal feature of their operations. Briefly, the method consists of the rinsing of all surfaces of utensils and other possible sources of contamination with aseptically drawn milk and the carrying out of methylene blue reduction tests on samples of the rinsings in order to obtain an index of the degree of contamination.

DETAILS OF THE METHOD.

Aseptic Withdrawal of Milk.

The amount of milk required for rinsing purposes depends on the dairy equipment to be examined. For farms not equipped with milking machines, 2,000 ml. are sufficient, but where milking machines are in use an additional 1,000 ml. should be drawn. It is not always possible to obtain the required quantity from one cow; invariably two cows must be milked when milking machines are in use.

The animals selected to provide the rinsing milk should preferably be in early lactation; they should have no udder or teat abnormalities, such as swollen glands, fibrosis, and sores; and the milk from each quarter should be free from clots, slime, blood and bad odour. Prior to drawing the milk the udder, teats and flanks are washed with clean water and the surplus moisture removed with a clean, squeezed-out cloth; the teats, udder and milker's hands are then thoroughly rubbed with cotton wool soaked in a chlorine solution containing 1,000 p.p.m. available chlorine, and excess chlorine solution removed on a squeezed-out cotton wool pad.

After the rejection of the first 8 to 10 streams of milk from each quarter, the milk is drawn into a sterile narrow-necked flask, such as a 2,000 ml. Erlenmeyer flask previously plugged with cotton wool and autoclaved. During milking the cotton wool plug is kept in a sterile jar and replaced immediately after drawing the requisite amount of milk. Strippings are excluded.

Rinsing of Surfaces of Utensils.

When rinsing utensils, care should be taken that the rinsings are made with the surfaces of the utensils in their normal condition. This may be achieved by requiring the dairy operator to make all equipment ready for use by his usual methods prior to performing the rinsing.

In dispensing the aseptically drawn milk a graduated cylinder of 250 ml. capacity is used. This is sterilized before use with a chlorine solution containing 1,000 p.p.m. available chlorine. Rinsing the cylinder with a small portion of the aseptically drawn milk or sterile water removes the greater portion of the residual chlorine. In experimental work both the milk container and the cylinder are covered with sterile petri dish lids to prevent air-borne contamination, but under practical conditions in the field this could probably be accomplished by using clean cups or tumblers as covers, and possibly cotton wool filter pads would suffice.

The following quantities of milk are considered to be suitable for the rinsing of various utensils:—

Milking machine	1,000 ml.
Vat	200 ml.
Can (8 or 10 gallon capacity)	200 ml.
Milking bucket	100 ml.
Strainer	200 ml.
Cooler	200 ml.

In flushing milking machines, a volume of approximately 1,000 ml. of milk is placed in a previously chlorinated bucket, from where it is drawn up through the milking unit most distant from the releaser. The milk used for flushing is collected from the releaser in a sterile container. When rinsing vats,

cans and buckets, it is necessary to tilt and roll each vessel as much as possible so that the milk rinse flows over all surfaces with which milk would normally come in contact.

From each rinsing a 10 ml. sample is retained for testing.

Sampling of Other Sources of Contamination.

Milk may be contaminated on the farm from sources other than dairy utensils. Manure and feed dusts, water, milkers' hands, and various articles of equipment, such as rags and brushes, all present a potential menace to the production of clean milk. These are sampled as follows:—

Manure and Feed Dust:—A 50 ml. sample of aseptically drawn milk is exposed for five minutes in a petri dish placed on the floor. The dish is then closed prior to withdrawing a portion for testing.

Water:—In the examination of water it has been usual to inoculate 1/10 ml. into the 10 ml. of milk required for the methylene blue test, but this quantity may need modification.

Rags and Brushes:—Rags and brushes are used in the dairy for washing or wiping udders and in some instances for wiping utensils. A sample for an estimate of the contamination provided by a rag can be obtained by squeezing out the rag, thoroughly wiping the inside of a previously sterilized and rinsed container—such as a wide-mouthed metal can—and then rinsing the container with 50 ml. of milk. A similar procedure is adopted for brushes, the container being brushed out before rinsing.

Milker's Hands.—These are examined by having the milker rinse his hands in 200 ml. of the aseptically drawn milk, after which a portion of the milk is taken for testing.

RESULTS OF EXAMINATIONS.

For the purpose of demonstrating the utility of the method in ascertaining sources of milk contamination, the results obtained on a number of farms are set out in the appendix. In addition to methylene blue reduction times the plate counts before and after laboratory pasteurization are given for each sample. It will be seen, for instance, that the kerosene tin and Can A sampled on Farm No. 1 carried a bacterial flora which gave the milk a low reduction time. On both Farms Nos. 2 and 3 the milking machine was obviously contaminated, whereas the milking machine on Farm 4 was relatively clean. It is of interest to note, too, how the results reflect the degree of cleanliness of the milker's hands.

DISCUSSION OF METHOD.

The procedure adopted in using the method under experimental conditions can be modified to meet the requirements of field advisory officers. In the experimental work the methylene blue test has been commenced within two hours of sampling, but where it is not practicable to test samples on the day of sampling they can be held until the following day under cool conditions

without nullifying the effects of contamination. Such a modification would enable several farms to be sampled on the one day, and would result in lower reduction times with a consequent saving in the time required for completing the tests (Smythe, 1945). As each farm is treated as a unit, the results obtained from the several farms sampled on the same day will not necessarily be comparable.

The resazurin test could be substituted for the methylene blue test, thus yielding quicker results, but at the present time resazurin is too costly and too difficult to procure in Queensland to permit its substitution for methylene blue.

Where equipment for methylene blue testing is not available to field officers, a simple keeping quality test could be employed. This is a test which could, in fact, be performed by the dairymen himself with the minimum of equipment.

ACKNOWLEDGMENT.

The authors wish to gratefully acknowledge the field assistance given in this work by Mr. F. C. Coleman, of the Division of Dairying.

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APPENDIX.

DATA FROM FIVE FARMS.

Surface, &c., Sampled.	Sampling Treatment.	Methylene Blue Reduction Time (hours).	Plate Count per ml.	
			Raw.	After Laboratory Pasteurization 63°C. for 30 mins.
	FARM NO. 1.			
Control milk, drawn aseptically	..	9¼	30	4
Kerosene tin	Milk rinse, 100 ml. ..	4½	32,000	800
Can A	Milk rinse, 200 ml. ..	4¾	33,000	500
Can B	Milk rinse, 200 ml. ..	5¾	5,000	600
Can C	Milk rinse, 200 ml. ..	6¾	..	1,900
Cooler	Milk rinse, 200 ml. ..	7¼	300	25
Udder cloth	Sterile tin wiped with cloth, then rinsed with 50 ml. milk	5¾	4,500	20
Utensil brush	Milk rinse, 50 ml. ..	6¾	12,000	700
Milker A's hands immediately after washing udder	Milk rinse, 200 ml. ..	5¼	9,000	100
Milker A's hands half-way through milking one cow	Milk rinse, 200 ml. ..	5	46,000	130
Milker B's hands immediately after washing mud-coated udder	Milk rinse, 200 ml. ..	5¼	5,000	180
Air on feed platform ..	50 ml. milk exposed for 5 mins.	7¼	400	6
Air under cow being milked	50. ml. milk exposed for 4 mins.	7½	1,500	4
Air in bails	50 ml. milk exposed for 5 mins.	6½	730	20
	FARM NO. 2.			
Control milk A, drawn aseptically	..	9	1,000	0
Control milk B, drawn aseptically	..	9+	1,000	0
Milking machine ..	Milk A rinse, 1,700 ml. ..	3½	216,000	43,000
Can	Milk B rinse, 200 ml. ..	6½	47,000	8,000
Strainer	Milk B rinse, 100 ml. ..	7½	20,000	5,000
Vat	Milk B rinse, 200 ml. ..	9+	5,700	0
Cooler	Milk B rinse, 100 ml. ..	8½	84,000	6,500
Udder cloth	Sterile tin wiped with cloth, then rinsed with 50 ml. milk B	8½	336,000	5,500
Milker A's hands ..	Milk B rinse, 200 ml. ..	6¼	260,000	1,400
Milker B's hands ..	Milk B rinse, 200 ml. ..	7½	184,000	5,000
Tank water	1/10 ml. in 10 ml. milk B	9+	3,000	..
Air under cow being milked	50 ml. milk B exposed for 5 mins.	9+	1,000	5
Air in bails	50 ml. milk B exposed for 5 mins.	9+	1,700	0
Feed dust	50 ml. milk B exposed for 5 mins.	9+	1,200	5

DATA FROM FIVE FARMS—*continued.*

Surface, &c., Sampled.	Sampling Treatment.	Methylene Blue Reduction Time (hours).	Plate Count per ml.	
			Raw.	After Laboratory Pasteurization 63°C. for 30 mins.
FARM NO. 3.				
Control milk A, drawn aseptically	..	9+	350	5
Control milk B, drawn aseptically	..	8¾	550	5
Milking machine ..	Milk A rinse, 1,700 ml. ..	2¼	2,000,000	58,000
Can	Milk B rinse, 200 ml. ..	7¼	53,000	20,000
Strainer	Milk B rinse, 100 ml. ..	9	1,500	150
Vat	Milk B rinse, 200 ml. ..	8	8,800	50
Cooler	Milk B rinse, 100 ml. ..	8¾	3,000	20
Udder cloth	Sterile tin wiped with cloth, then rinsed with 50 ml. milk B	7¾	900	25
Vat rag	Sterile tin wiped with cloth, then rinsed with 50 ml. milk B	8½	900	35
Milker's hands	Milk B rinse, 200 ml. ..	5½	25,000	750
Tank water	1/10 ml. in 10 ml. milk B	9	1,400	..
Air under cow being milked	50 ml. milk B exposed for 5 mins.	8½	580	10
Air in bails	50 ml. milk B exposed for 5 mins.	9	750	5
FARM NO. 4.				
Control milk A, drawn aseptically	..	..	55	..
Control milk B, drawn aseptically	..	9½+	100	5
Milking machine ..	Milk A rinse, 1,500 ml. ..	8	3,200	2,000
Can A	Milk B rinse, 200 ml. ..	6½	28,000	24,000
Can B	Milk B rinse, 200 ml. ..	5¼	200,000	21,000
Udder cloth A	Sterile tin wiped with cloth, then rinsed with 50 ml. milk B	9½	300	0
Udder cloth B	Sterile tin wiped with cloth, then rinsed with 50 ml. milk B	9½+	370	10
Milker A's hands ..	Milk B rinse, 200 ml. ..	5½	32,000	3,400
Milker B's hands ..	Milk B rinse, 200 ml. ..	5¼	37,000	..
Tank water	1/10 ml. in 10 ml. milk B	9	1,400	..
Air under cow being milked	50 ml. milk B exposed for 5 mins.	8½	4,500	500
Air in bails	50 ml. milk B exposed for 5 mins.	9	140	0
Bulk milk entering can	..	6½	45,000	7,000

DATA FROM FIVE FARMS—*continued.*

Surface, &c., Sampled.	Sampling Treatment.	Methylene Blue Reduction Time (hours.)	Plate Count per ml.	
			Raw.	After Laboratory Pasteurization 63°C. for 30 mins.
FARM NO. 5.				
Control milk drawn aseptically	..	9+	45	0
Can A	Milk rinse, 200 ml. ..	7½	84,000	33,000
Can B	Milk rinse, 200 ml. ..	6½	81,000	20,000
Bucket	Milk rinse, 100 ml. ..	6½	11,400	100
Vat and cooler	Milk rinse, 200 ml. ..	9+	400	20
Udder cloth	Sterile tin wiped with cloth, then rinsed with 50 ml. milk	5¼	7,000	150
Milker's hands immediately prior to beginning milking	Milk rinse, 200 ml. ..	5	52,000	150
Tank water	1/10 ml. in 10 ml. milk	9+	10,000	..
Air under cow being milked	50 ml. milk exposed for 5 mins.	8	250	20
Air in bails	50 ml. milk exposed for 5 mins.	7	9,000	10

WATERY-WHITENESS OF MARKET EGGS.

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SUMMARY.

1. It was found impossible to measure the viscosity of thick egg albumen by the methods employed. Thin albumen, however, was found to be just as viscous in watery-white eggs as in those of first quality.

2. The defect appears to be confined to the apparent thick albumen only.

3. Watery-white eggs kept almost as well as first quality over a 14-day period. The differences were not large enough to warrant the inclusion of watery-white eggs with second quality eggs, whose keeping quality was very poor.

4. No evidence of bacterial infection was found in watery-white eggs, and efforts to transfer the defect to normal eggs using the watery-white albumen as inoculum were unsuccessful.

INTRODUCTION.

An ideal egg is generally taken to be one having a clean shell, possessing desirable size and shape and, on breaking, exhibiting a firm round yolk well supported by a considerable amount of apparent thick albumen. Its appearance, odour and flavour must be attractive. Any abnormality must, of course, be expressed in terms of departure from this ideal. A watery-white egg exhibits a weakening of the albumen as a whole, but more particularly a lack of firmness of the apparent thick albumen. On candling, the defect seems to result not only in a more freely mobile yolk than usual, but frequently also in a movable air cell. Queensland Egg Board graders report that the defect is often associated with a large air cell, which is usually an indication of "staling" of an egg. On breaking a watery-white egg, the thick albumen instead of supporting the yolk tends to spread itself away from the yolk.

Queensland records concerning the incidence of the defect are unobtainable. Some graders report that in many instances a very high percentage of the eggs graded as second grade has been watery-white. There can be no doubt, however, that the trouble is of frequent occurrence, particularly during hot weather. The defect does not appear to be confined to aged eggs. One of us (P.R.) has noticed the defect in eggs immediately after they were laid. Anderson (1938) has also reported that some eggs are laid as watery-whites. The question is raised whether the defect is confined to a weakness of the envelope of thick albumen. It appears likely that a weakness or rupture of the thick envelope would cause an apparent watery condition.

This investigation was carried out during February, 1944. Primarily the answers to two questions were sought:

(1) Is the keeping quality of watery-white eggs so inferior to that of first quality that their grading as second quality is warranted on this defect alone?

(2) Is watery-whiteness of bacterial origin, being transferable from one egg to another?

GENERAL CONSIDERATIONS.

Candling as a Measure of Egg Quality.

There can be no doubt that candling is at present the only satisfactory method of grading eggs for a general market, but the very indirectness of the method itself places emphasis on the need for great care in the interpretation of candling observations. Hoover (1938) found a lack of correlation between candling grade and the interior quality of eggs, while in Australia Anderson (1936) has stated: "It is almost impossible to get two graders to grade alike. . . . . This is one of the principal difficulties confronting the egg trade. No form of mechanical testing for quality appears feasible at this juncture."

Albumen Quality.

Considerable interest has centred round the study of the albumen of eggs and factors which affect albumen quality. The percentage of thick albumen in eggs is a heritable characteristic, being a feature of the individual hen (Knox and Godfrey, 1938). The effect of inheritance with respect to albumen quality has also been studied by Wilhelm and Carver (1940 a and b) and Evans and Carver (1942). There is a distinct seasonal effect upon the percentage of thick albumen (Hunter *et al.*, 1936; Knox and Godfrey, 1938; and Wilhelm and Heiman, 1938). The amount of firm albumen has no bearing on its quality (Van Wagenen and Wilgus, 1935). Eggs rapidly lose albumen quality after they are laid. Besides temperature and humidity a time factor may be responsible for such deterioration (Wilhelm, 1939). Conrad and Scott (1939) have found that the wateriness of albumen produced by storage is not due to any enzymatic hydrolysis of the mucin present; in fact, if any decrease in the amount of mucin occurs it must be due simply to the effect of pH. The microscopic structure of the gel is unchanged, so that the change in properties must be due to a change in elasticity of the mucin fibres. The decrease in pH plays a very important part in this change. According to Wilhelm and Heiman (1938) albumen quality is unaffected by environmental temperature of the laying stock. Temperature of transport and storage, however, does affect albumen quality (Anderson, 1936; Wilhelm and Carver, 1940b; Evans and Carver, 1941; and Brant, 1943) and may increase the percentage of watery-whites (Anderson, 1936). Humidity of storage, according to Wilhelm and Carver (1940b), also affects albumen quality, but in contradiction of this Evans and Carver (1941) found that the relative humidity had little effect on the albumen index at a given temperature.

Tremulous Air Cells.

Hoover and Rogers (1939) have shown that both the candling process itself and several hours of transport cause a lowering in grade. It must be presumed that this lowering in grade results from the presence of tremulous air cells, since the evidence of other workers (Almquist *et al.*, 1934; Knox and Olsen, 1936; and Wilhelm, 1939) leads to the belief that rough handling will produce tremulous air cells but leave the albumen quality unaffected. Stidston (1939) stated: "The term 'watery-white' is often regarded as synonymous with the term 'tremulous air cell.' Watery-white does not necessarily mean that the egg has a small amount of thick white present. They are usually determined by observation of the air cell in candling and of its behaviour on rotating the egg."

All first quality eggs (6 dozen) used in the experimental work to be discussed were entirely free from tremulous air cells, but such air cells were present in both second quality and watery-white eggs. This rather suggests that all eggs showing air cells departing in any degree from the rigid condition are discarded from the first quality grade.

Yolk Shadow.

One other aspect of candling—namely, yolk shadow—is worthy of mention. A perusal of Appendix 2 will show that every first quality egg exhibited a pale yolk, while the yolk of every second quality egg was dark. It is reasoned that this yolk colour has a profound influence on the visibility of the yolk in candling. The work of other investigators (Parker *et al.*, 1926; Parker, 1927; Stewart *et al.*, 1932; Almquist, 1933; and Sharp, 1937) substantiates this claim. Van Wagenen and Wilgus (1935), however, found a significant correlation between yolk visibility in candling and observed albumen quality. As the yolk shadow appeared more visible and mobile in candling the score of the condition of the firm albumen was poorer. The following table from Almquist (1933) shows the relation of yolk colour to yolk shadow.

Colour of Yolk.	Light Yolk Shadow.	Distinct Yolk Shadow.
	%	%
Pale	76	24
Medium	53	47
Dark	23	77

The question can be raised here, Is there any correlation between the uniformly dark yolks of second quality eggs and their inferior keeping quality? It may be true, however, that most eggs having dark yolks are produced in country centres from birds with access to range. In addition the distance from market would create a slight age handicap when graded at the Egg Board's premises in Brisbane.

EXPERIMENTAL.

The Viscosity of Watery-white Eggs.

Before any investigational work was done it was thought advisable to obtain some measure of the viscosity of egg albumen in order that the extent of wateriness might be expressed numerically. This proved difficult. Eggs commercially graded into first quality and watery-whites were brought into the laboratory and attempts made to measure the albumen viscosity. Initially an attempt was made to determine the rate of flow of liquid paraffin through the albumen, but this was unsuccessful despite the range of mixtures of liquid paraffin and kerosene employed. Following this a Hoppler Viscosimeter was procured.

This instrument permits the measurement of the rate of fall of a standard ball through an inclined column of the medium. The absolute viscosity in centipoises can be calculated simply from the fall time, the constants of the instrument and the density of the medium.

Egg albumen was separated by means of a wire gauze into thick and thin portions, but owing to the ability of the thick albumen to retain some of the less dense portion within its mucilaginous "envelope" the separation was never complete. As a result of this it was impossible to obtain readings with the thick albumen, the ball travelling in a jerky instead of a smooth manner.

With both thick and thin albumen it was found that the period of fall decreased rapidly with each successive attempt to obtain a reading. This was apparently due to the denaturation of the albumen in passing through the narrow gap between the ball and the tube wall. Readings were obtained with thin albumen but only the first two agreed to any extent. At least three eggs had to be broken before sufficient albumen was obtained to fill the tube. The results of these viscosity measurements are given in Table 1, and the relative quantities of thick and thin albumen for each egg are recorded in Table 2.

Table 1.

VISCOSITY MEASUREMENTS OF FIRST QUALITY AND WATERY-WHITE EGGS.

Grade.	Measurements with Hoppler. Viscosimeter on thin Albumen only.	
	Time of Fall in Seconds using Ball No. 2.	Approximate Viscosity in Centipoises assuming the Density of Albumen = 1. (Initial reading only taken).
First Quality (Thin albumen only)	Reading 1 20·0 ,, 2 19·1 ,, 3 18·9 ,, 4 18·0	5·3
Watery-whites (Thin albumen only)	Reading 1 20·0 ,, 2 19·0 ,, 3 16·3 ,, 4 15·2	5·3

It will be seen that the period of fall decreased more rapidly in the case of watery-white eggs, but the significance of this is not clear. In other respects the figures show the thin albumen of the two grades to be similar, which suggests that the defect is confined to the thick albumen only. The same result is shown by the figures for the relative quantities of thick and thin albumen given in Table 2.

Table 2.

Relative Amounts of Thick and Thin Albumen in First Quality and Watery-white Eggs.

Grade.	Amount of Albumen per Egg in c.c.		
	Thick.	Thin.	Total.
First Quality	15	13	28
	12	14	26
	17	15	32
	18	12	30
Watery-whites	19	15	34
	11	13	24
	18	17	35

Because of the difficulties associated with its use, and the limited results obtained with it, the Hoppler Viscosimeter was deemed unsuitable for routine determinations of albumen viscosity. Further attempts at measuring this factor were abandoned.

Wilcke (1936) used the torsion pendulum to measure the viscosity of the contents of eggs without breaking the shell. His method measures the composite viscosity of all egg contents and not of any one component. His results lead to the conclusion that total egg viscosity could not be correlated with percentage volume or viscosity of the thin white nor with the volume of the thick white. Furthermore, the total egg viscosity is a characteristic of the individual hen and is not affected by feeding.

In the following work the appearance of the egg on candling and on breaking was used as the sole measure of its quality.

The Keeping Quality of Graded Eggs.

Twelve dozen graded eggs were obtained from the Queensland Egg Board. These eggs comprised four dozen first quality, four dozen watery-whites, and four dozen second quality exclusive of watery-whites. On arrival at the laboratory all eggs were candled, and one of each class was broken to observe the condition of the contents. An equal number of eggs from each class was stored at three temperatures—99°F., 75-80°F. and 50-55°F.—representing high temperature, summer room temperature and refrigerator temperature respectively. The temperature of the 99°F. incubator remained constant, and the temperature inside the refrigerator and the incubator at room temperature fluctuated within the above limits.

All eggs were candled and some were broken at regular intervals throughout the 14 days storage trial. Eggs were held with large end always upwards. Tables 3 and 4 give summaries of each lot of results; details of the examinations appear in Appendices 1 and 2.

Table 3.

SUMMARY OF CANDLING EXAMINATIONS.

Treatment.	Observations.
Refrigerator, 50–55° F. ..	All 3 grades of eggs kept equally well over 14 days
Room, 75–80° F.	Water-whites kept as well as 1st quality over 10 days, but 1 watery-white was bad after 14 days. Second quality deteriorated rapidly, all 2nd quality being unsaleable after 10 days
Incubator, 99° F.	Watery-whites kept equally as well as 1st quality. 2nd quality deteriorated rapidly. More cases of germ mortality occurred in watery-white eggs than in the other two groups.

Table 4.

SUMMARY OF BREAKING EXAMINATIONS*.

Treatment.	Observations.
Refrigerator, 50–55° F. ..	All eggs kept well up to 14 days, but 1st quality eggs were slightly better than watery-whites and watery-whites were better than 2nd quality
Room, 75–80° F.	Keeping quality of watery-whites was equal to that of 1st quality. 2nd quality were inferior after 7 days
Incubator, 99° F.	Watery-whites kept equally as well as 1st quality, but all eggs were unfit for retail sale after 7 days

* One egg from each group was broken and examined on each occasion.

It would appear from the results given in Tables 3 and 4 that, whilst second quality eggs are definitely inferior to first quality after storage at any temperature, this is hardly true in the case of watery-whites. In odd instances only did watery-whites deteriorate more rapidly than first quality and always the difference was slight. Anderson (1936) stated that eggs slightly affected with "watery-white" should not be graded out as of second quality.

Bacterial Examinations of Watery-white Eggs.

Suggestions have been put forward that the watery nature of the albumen may be of bacterial origin, either in the manner of a diseased condition of the laying stock or a bacterial infection of the egg. An examination of the relevant literature has shown that the bacterial content of eggs varies greatly and would appear to be dependent on a great many factors (Pennington *et al.*, 1914; and Haines and Moran, 1940). However, the general impression that can be gleaned

is that a perfectly fresh egg produced by a healthy fowl under ideal conditions of cleanliness is usually sterile. Contamination after production may, however, be considerable, depending on the condition of the egg, particularly the shell, and on general conditions of environment. Haines and Moran (1940) found that bacterial infection of an egg through the shell is most likely to occur immediately after laying. In this state while the egg contents are cooling and contracting, resulting in the formation of the air cell, the inward suction is conducive to infection through the pores of the shell, particularly when the egg is dropped into moist and unclean nesting material. When the shell is cracked contamination is made still easier, such cracked eggs being most prone to internal moulding.

Whether or no the defect is the result of a diseased condition of the laying hens was not investigated. The bacterial condition of watery-white eggs was, however, examined.

The albumen from eggs graded as watery-white by the Queensland Egg Board was examined bacteriologically, and was found to be sterile to milk agar after aerobic incubation for 3 days at 37°C. An attempt was made to transfer watery-whiteness to sound fresh eggs, using "watery-white" albumen (0·1 ml.) as inoculum, but was unsuccessful. After 3 days at 37°C. the inoculated eggs were also sterile. It is concluded from these results that watery-whiteness is not the direct result of bacterial activity within the egg, despite the fact that albumen only was used as inoculum. It is considered highly improbable that bacteria confined entirely to the yolk would produce any thinning of the albumen and this the only change. The fact that the defect is known to occur in eggs immediately after laying adds strength to the belief that it is not bacterial in origin.

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APPENDIX 1.

DETAILS OF CANDLING OBSERVATIONS.

No. Days.	Temp. °F.	First Quality.	Second Quality excluding Watery-whites.	Watery-whites.
0	..	No. of eggs 48. All sound	No. of eggs 48, 36 clean, 12 soiled. Prominent yolks and large air cell—35 (7 soiled). Large air cell—10 (2 soiled). Soiled only—3 (3 soiled)	No. of eggs 48. Moveable air cell and apparently watery, 36. Fixed air cell (wateriness not readily discernible)—12
3	50–55	No change	No change	No change
	75–80	No change	1 egg showing deterioration	No change
	99	Yolks inclined to rise	1 egg definitely bad (discarded). Yolks more prominent in all cases	Yolks inclined to rise. (No greater change than 1st quality)
7	50–55	No change	No change	No change
	75–80	No change	2 more eggs showed yolks slightly more pronounced.	No change
	99	5 showing germ development (discarded) 3 of which alive; remaining 6 eggs sound	8 showing germ development (discarded); 7 alive; remaining 2 eggs sound	3 showing dead germ. All others equal to 1st quality
10	50–55	1 yolk rising	One yolk very high ..	One yolk very high
	75–80	Yolks elevated ..	Yolks very elevated. Have reached limit as a saleable egg	Yolks elevated
	99	2 discarded; 4 remaining showing some deterioration	Remaining 2 eggs discarded	All but 5 eggs discarded
14	50–55	1 yolk very high; all air cells slightly enlarged	One yolk very high; all air cells slightly enlarged	One yolk high; all air cells slightly enlarged
	75–80	All yolks very high ..	Deterioration very marked. 1 bad	All yolks very high. 1 bad
	99	All eggs discarded ..	..	All eggs discarded

APPENDIX 2.

Details of Breaking Observations.

One egg from each group was broken and examined on each occasion.

No. Days.	Temp. °F.	1st Quality			2nd Quality Excluding Watery-whites.			Watery-whites.		
		Yolk.	Albumen.	Colour.	Yolk.	Albumen.	Colour.	Yolk.	Albumen.	Colour.
0	..	High	Firm	Yellow	High	Slightly weak	Orange	High	Slightly weak	Yellow-orange
3	50–55	High	Firm	Yellow	Slightly inclined to flatness	Weak	Orange	Slightly inclined to flatness	Slightly weak	Yellow
	75–80	High	Slightly weak	Yellow	ditto	Weak	Orange	ditto	Weak	Yellow
	99	Flattened	Weak	Yellow	Flattened	Weak	Orange	Flattened	Weak	Yellow
7	50–55	High	Firm	Yellow	High	Firm	Orange	High	Firm	Yellow
	75–80	Slightly flat	Slightly weak	Yellow	Slightly flat	Slightly weak	Orange	Slightly flat	Slightly weak	Yellow
	99	Flat	Very weak	Yellow	Flat	Very weak	Orange	Flat	Very weak	Yellow
All eggs at high temperature had reached the stage of being unfit for retail sale.										
10	50–55	High	Firm	Yellow	High	Weak	Orange	High	Firm	Yellow
	75–80	Slightly flat	Fairly firm	Yellow	Flat	Weak	Orange	Flat	Very weak	Yellow
	99	Very flat	Very weak	Yellow	All eggs bad			Very flat	Very weak	Yellow
14	50–55	High	Firm	Yellow	High	Weak	Orange	High	Weak	Yellow
	75–80	Flat	Weak	Yellow	Flat	Weak	Orange	Flat	Weak	Yellow
	99			All eggs bad						

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